

ON-35 5' TC'TC'TC'TC'TC'T'T'T'T'T' 3' (SEQ ID NO:43)

ON-36 5' TC'TC'TC'TC'TC'U\*U\*U\*U\*U\* 3' (SEQ ID NO:44)

ON-37 5' TC'TC'TC'TC'TC'U<sup>x</sup>U<sup>x</sup>U<sup>x</sup>U<sup>x</sup> 3' (SEQ ID NO:45)

ON-38 5' TC''TC''TC''TC''TC''TTTTT 3' (SEQ ID NO:46)

ON-39 5' TC\*TC\*TC\*TC\*TC\*TTTTT 3' (SEQ ID NO:47)

ON-40 5' TC<sup>x</sup>TC<sup>x</sup>TC<sup>x</sup>TC<sup>x</sup>TC<sup>x</sup>TTTTT 3' (SEQ ID NO:48)

21. On page 108, please delete the paragraph (line 30) and replace with the following amended paragraph:

**RNA Target:** 5' AAAAAGAGAGAGAGA 3' (SEQ ID NO: 49)

22. On page 110, please delete the paragraph (lines 1 through 7) and replace with the following amended paragraph:

ON-41 5' U\*C\*U\*C\*U\*U\*U\*U\*U\*U\*C\*U\*U\*C\*U\*C\*U\*U\*U\*C\*X\*U\*U\*U\*U\*U\*U\*U\*U\* 3' (SEQ ID NO:50)

ON-42 5' U\*C'U\*C'U\*U\*U\*U\*U\*U\*C'U\*U\*C'U\*C'U\*U\*U\*C'X\*U\*U\* 3' (SEQ ID NO:51)

The DNA target sequence used was:

**DNA Duplex Target:** 5' AGAGAAGGGAGAAGAGAAAGAAATTTTTTTT 3'(SEQ ID NO:52)  
3' TCTCTTTTTCTTCTCTTCTTTAAAAAAA 5'(SEQ ID NO:53)

## REMARKS

Claims 128-138 are pending. The specification has been amended to incorporate the sequence listing submitted herewith. No new matter has been added. Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is entitled **“Version with Markings to Show Changes Made.”**


The foregoing constitutes a complete response. Applicants submit that this

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**PATENT**

application is now in condition for allowance. Accordingly, an indication of allowability and an early Notice of Allowance are respectfully requested.

Respectfully submitted,



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Date: **June 18, 2002**

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VERSION WITH MARKINGS TO SHOW CHANGES MADE**In the Specification**

1. On page 82, please delete the paragraphs (lines 5 through 34) and replace with the following amended paragraph:

Example 2

Formation of Triple Helix Structures Using  
Oligomers (ON) Containing 5-Propynyl Uracil  
Residues that Bind to Duplex DNA

Three oligomers were synthesized as follows:

ON-1 5'TC'TC'TC'TC'TC'TTTTT 3' (SEQ ID NO: 1)

ON-2 5'TC'TC'TC'TC'TC'U\*U\*U\*U\*U\* 3' (SEQ ID NO:2)

ON-3 5' TC'TC'TC'U\*C'U\*C'U\*U\*U\*U\* 3' (SEQ ID NO:3)

Each oligomer (ON) was hybridized with duplex DNA containing the target sequence 5' AGAGAGAGAGAAAAA 3' (SEQ ID NO:4). Hybridization was carried out in 140 mM KCl, 5 mM MgCl<sub>2</sub>, 5 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 6.6. Thermal stability, T<sub>m</sub>, of the resulting triple helix formed between each oligomer and the target sequence was determined. The following T<sub>m</sub> values were obtained, ON-1 (control oligomer) was 42.1 °C, ON-2 was 48.1 °C and ON-3 was 55 °C. The increased T<sub>m</sub> values of ON-2 and ON-3 were not expected and demonstrated that the triple helix formed was more stable than the corresponding control triple helix structure.

Example 3Binding of Oligomers Containing 5-PropynylUracil or 5-Propynyl CytosineTo Single-Stranded RNA

Oligomers were synthesized as follows:

ON-1 5'TC'TC'TC'TC'TC'TTTTT 3' (SEQ ID NO: 1)

ON-3 5' TC'TC'TC'U\*C'U\*C'U\*U\*U\*U\* 3' (SEQ ID NO:3)

ON-4 5' TC\*TC\*TC\*TC\*TC\*TTTTT 3' (SEQ ID NO: 5)

2. On page 83, please delete the paragraphs (lines 1 through 24) and replace with the following amended paragraph:

The oligomers were hybridized with a single-stranded target RNA sequence, 5' AAAAAGAGAGAGAGA 3' (SEQ ID NO:6), in 140 mM KCl, 5 mM MgCl<sub>2</sub>, 5 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 6.6. The following T<sub>m</sub> values for the duplexes were obtained; ON-1 (control) was 65.5 °C, ON-3 was 74.0 °C and ON-4 was 73.0 °C. Duplexes formed with ON-3 and ON-4 were more stable than the control oligomer. Surprisingly, ON-3 and ON-4 gave increased T<sub>m</sub> values which demonstrated that the duplex formed was more stable than the corresponding control duplex structure.

Example 4Formation of Triple Helix Structures at Elevated pH

Triple helix formation at elevated pH was demonstrated using ON-1 as a control and ON-5, 5' U\*C'U\*C'U\*C'U\*C'U\*C'U\*U\*U\*U\*U\* 3' (SEQ ID NO:7). Oligomers were hybridized with duplex target DNA, as described in Example 2 except that the buffer was at pH 7.4. T<sub>m</sub> values of the triple helix were then determined. ON-1 had a T<sub>m</sub> of 27.1 while ON-5 had a T<sub>m</sub> of 51.5. Thus, oligomers containing 5-propynyl uracil were capable of triplex formation at high pH levels, while the control oligomer formed triplex structure only at temperatures that are below physiological.

3. On page 84, please delete the paragraph (lines 26 through 34) and replace with the following amended paragraph:

ON-1 5'TC'TC'TC'TC'TC'TTTTT 3' (SEQ ID NO: 1)

ON-6 5' TC'TC'TC'U'C'U'CU'TU'TU' 3' (SEQ ID NO: 8)

ON-7 5' TC'TC'TC'TC'TC'U'U'U'U'U' 3' (SEQ ID NO:9)

Base residues designated U' correspond to 5-(3-methyl-1-butynyl)uracil. The oligomers were hybridized with duplex DNA containing the target sequence, 5' AGAGAGAGAGAAAAA 3' (SEQ ID NO:4).

4. On page 85, please delete the paragraph (lines 15 through 17) and replace with the following amended paragraph:

**DNA Duplex Target:** 5' AGAGAGAGAGAAAAAGGA<sup>T</sup> T (SEQ ID NO:10)

3' TCTCTCTCTCTTTTTCCT<sub>T</sub> T (SEQ ID NO:11)

**RNA Target:** 5' AAAAAGAGAGAGAGA 3' (SEQ ID NO:12)

5. On page 85, please delete the paragraph (lines 27 through 29) and replace with the following amended paragraph:

ON-8 5' TC'TC'TC'U\*C'U\*C'U\*U\*U\*U\* 3' (SEQ ID NO:3)

ON-9 5' TC\*TC\*TC\*TC\*TC\*TTTTT 3' (SEQ ID NO: 5)

ON-10 5' U\*C\* U\*C\* U\*C\* U\*C\* U\*C\*U\*U\*U\*U\* (SEQ ID NO: 54)

6. On page 88, please delete the paragraph (lines 5 through 9) and replace with the following amended paragraph:

ON	ON Linkage		
	Diester	Thioate	$\Delta T_m$
ATTTTC'TTC'ATTTTTC'TTC'(SEQ ID NO:14)	54.0	40.0	-14.0

AU*U*U*U*C'U*U*C'AU*U*U*U*U*U*C'U*U*C' (SEQ ID NO:15)	76.5	68.5	-8.0
AU*U*U*U*C*U*U*C*AU*U*U*U*U*U*C*U*U*C* (SEQ ID NO:16)	82.5	76.5	-6.0

7. On page 88, please delete the paragraph (lines 18 through 19) and replace with the following amended paragraph:

ON-21: AU\*U\*U\*U\*C'U\*U\*C'AU\*U\*U\*U\*U\*U\*C'U\*U\*C' (SEQ ID NO: 17)

ON-22: U\*U\*AU\*U\*AU\*C'U\*U\*C'U\*U\*C'U\*U\*U\*U\*C'U\* (SEQ ID NO:25)

8. On page 89, please delete the paragraph (lines 15 through 24) and replace with the following amended paragraph:

ON 11: 5' ATTTTC'TTC'ATTTTTTC'TTC'3' (SEQ ID NO:14)

ON 12: phosphorothioate form of ON-11

ON 13: 5'AU\*U\*U\*U\*C\*U\*U\*C\*AU\*U\*U\*U\*U\*U\*C\*U\*U\*C\* 3' (SEQ ID NO:16)

ON 14: phosphorothioate form of ON-13

ON 15: phosphorothioate 5' C\*U\*U\*C\*AU\*U\*U\*U\*U\*U\*C\*U\*U\*C\* 3' (SEQ ID NO: 18)

ON 16: phosphorothioate 5' C\*U\*U\*C\*AU\*U\*U\*U\*U\*U\*C\*U\* 3' (SEQ ID NO: 19)

ON 17: phosphorothioate 5' C\*U\*U\*C\*AU\*U\*U\*U\*U\*U\* 3' (SEQ ID NO: 20)

ON 18: phosphorothioate 5' C\*U\*U\*C\*AU\*U\*AU\*U\*U\*C\*U\*U\*C\* 3' (SEQ ID NO: 21)

ON 19: phosphorothioate 5' C\*U\*U\*U\*C\*U\*U\*C\*U\*U\*AC\*U\*U\*C\* 3' (SEQ ID NO: 22)

9. On page 91, please delete the paragraph (lines 11 through 17) and replace with the following amended paragraph:

In addition to inhibition of TAg synthesis, a phosphorothioate oligomer, ON 20, 5' U\*U\*GC'C'GU\*U\*U\*U\*C'AU\*C'AU\*AU\*U\*U\*AAU\* 3' (SEQ ID NO:23), that is complementary to the  $\beta$ -galactosidase RNA, was able to inhibit  $\beta$ -galactosidase in a sequence specific manner with an  $IC_{50}$  of 0.25  $\mu$ m. ON 20A, ON 20 with T and C', did not inhibit  $\beta$ -galactosidase expression in a sequence specific manner.

10. On page 92, please delete the paragraph (lines 27 through 34) and replace with the following amended paragraph:

Target Sequence Binding and Target Gene Inhibition. An oligomer (5' ATTTTC'TTC'ATTTTTC'TTC' 3'(SEQ ID NO:14)) was systematically varied, using the phosphodiester antisense oligomer, ON-11, as a control. The phosphorothioate analog, ON-12, of the same oligomer was also prepared, but had no altered bases. The corresponding oligomer having the 5-substituted bases of

11. On page 93, please delete the paragraph (lines 16 through 20) and replace with the following amended paragraph:

Both the 9-mer (5' C\*U\*U\*C\*AU\*U\*U\*U\* 3' (SEQ ID NO:24)) and 11-mer (ON-17) phosphorothioates were able to inhibit T antigen synthesis when they contain the 5-substituted bases of the invention. However, the 9-mer had relatively weak sequence-specific inhibitory activity.

12. On page 93, please delete the paragraph (lines 28 through 29) and replace with the following amended paragraph:

5' UT oligomer: 5'-GCC TCC TCA CTA CTT CTG GA-3' (SEQ ID NO:26)

AUG oligomer: 5'-CAT CTT TGC AAA GCT TTT TG-3' (SEQ ID NO:27)

13. On page 104, please delete the paragraph (lines 9 through 13) and replace with the following amended paragraph:

ON-1 5' TC'TC'TC'TC'TC'TTTTT 3' (SEQ ID NO:1)

ON-23 5' TC'TC'TC'TC'TC'U\*U\*U\*U\*T 3' (SEQ ID NO:28)

ON-24 5' TC'TC'TC'TC'TC'T•TT•TT 3' (SEQ ID NO:29)

ON-25 5' TC'TC'TC'TC'TC'U\*•U\*U\*•U\*T 3' (SEQ ID NO:30)

ON-26 5' TC'TC'TC'TC'TC'U\*oU\*U\*oU\*T 3' (SEQ ID NO:31)

14. On page 104, please delete the paragraph (lines 21 through 23) and replace with the following amended paragraph:

**DNA Duplex Target:** 5' AGAGAGAGAGAAAAAGGA<sup>T</sup> T (SEQ ID NO:10)

3' TCTCTCTCTCTTTTCCT T (SEQ ID NO:11)

**RNA Target:** 5' AAAAAGAGAGAGAG 3' (SEQ ID NO:12)

15. On page 105, please delete the paragraph (lines 19 through 24) and replace with the following amended paragraph:

ON-1      5' TC'TC'TC'TC'TC'TTTTT 3' (SEQ ID NO:1)

ON-28 5' TC'TC'TC'TC'TC'U<sup>P</sup>U<sup>P</sup>U<sup>P</sup>U<sup>P</sup>U<sup>P</sup> 3' (SEQ ID NO:32)

ON-29 5' TC'TC'TC'U<sup>P</sup>C'U<sup>P</sup>C'U<sup>P</sup>TU<sup>P</sup>TU<sup>P</sup> 3' (SEQ ID NO:33)

ON-30 5' TC<sup>P</sup>TC<sup>P</sup>TC<sup>P</sup>TC<sup>P</sup>TC<sup>P</sup>TTTTT3' (SEQ ID NO:34)

ON-43      5' TC'TC'TC'TC'TC'U<sup>T</sup>U<sup>T</sup>U<sup>T</sup>U<sup>T</sup>U<sup>T</sup>3' (SEQ ID NO:35)

ON-44 5' TC'TC'TC'U<sup>T</sup>C'U<sup>T</sup>C'U<sup>T</sup>TU<sup>T</sup>TU<sup>T</sup>3' (SEQ ID NO:36)

16. On pages 105, please delete the paragraph (page 105, line 32 to page 106, line 1) and replace with the following amended paragraph:

**DNA Duplex Target:** 5' AGAGAGAGAGAAAAAGGA<sup>T</sup> T (SEQ ID NO:10)

3' TCTCTCTCTCTTTTCCT T (SEQ ID NO:11)

**DNA/RNA Target Sequence:** 5' AAAAAGAGAGAGAGA 3' (SEQ ID NO:12)

17. On page 106, please delete the paragraph (lines 29 through 32) and replace with the following amended paragraph:

ON-1            5' TC'TC'TC'TC'TC'TTTT 3' (SEQ ID NO:1)

ON-32 5' U\*C#U\*C#U\*C#U\*C#U\*U\*U\*U\*U\* 3' (SEQ ID NO:37)

ON-33 5' TTTMTTTMMTMMTTTTT 3' (SEQ ID NO: 38)

ON-34 5' U\*U\*U\*MU\*U\*U\*MMU\*MMU\*U\*U\*U\*U\* 3' (SEQ ID NO: 39)



18. On page 107, please delete the paragraph (lines 8 through 9) and replace with the following amended paragraph:

**DNA Duplex Target:** 5' AGAGAGAGAGAAAAA 3' (SEQ ID NO:4)  
3' TCTCTCTCTCTTTT 5' (SEQ ID NO:40)

19. On page 107, please delete the paragraph (lines 19 through 20) and replace with the following amended paragraph:

**DNA Duplex Target:** 5' AAAGAAAGGAGGAAAAA 3' (SEQ ID NO:41)  
3' TTTCTTTCCTCCTTTT 5' (SEQ ID NO:42)

20. On page 108, please delete the paragraph (lines 18 through 24) and replace with the following amended paragraph:

ON-1 5' TC'TC'TC'TC'TC'TTTT 3' (SEQ ID NO:1)  
ON-35 5' TC'TC'TC'TC'TC'T'T'T'T' 3' (SEQ ID NO:43)  
ON-36 5' TC'TC'TC'TC'TC'U\*U\*U\*U\*U\* 3' (SEQ ID NO:44)  
ON-37 5' TC'TC'TC'TC'TC'U<sup>x</sup>U<sup>x</sup>U<sup>x</sup>U<sup>x</sup>U<sup>x</sup> 3' (SEQ ID NO:45)  
ON-38 5' TC''TC''TC''TC''TC''TTTT 3' (SEQ ID NO:46)  
ON-39 5' TC\*TC\*TC\*TC\*TC\*TTTT 3' (SEQ ID NO:47)  
ON-40 5' TC<sup>x</sup>TC<sup>x</sup>TC<sup>x</sup>TC<sup>x</sup>TC<sup>x</sup>TTTT 3' (SEQ ID NO:48)

21. On page 108, please delete the paragraph (line 30) and replace with the following amended paragraph:

**RNA Target:** 5' AAAAAGAGAGAGAGA 3' (SEQ ID NO: 49)

22. On page 110, please delete the paragraph (lines 1 through 7) and replace with the following amended paragraph:

ON-41 5' U\*C'U\*C'U\*U\*U\*U\*U\*U\*C'U\*U\*C'U\*C'U\*U\*U\*C'X\*U\*U\*U\*U\*U\*U\* 3' (SEQ ID NO:50)

ON-42 5' U\*C'U\*C'U\*U\*U\*U\*U\*U\*C'U\*U\*C'U\*C'U\*U\*U\*C'X\*U\*U\* 3' (SEQ ID NO:51)

The DNA target sequence used was:

**DNA Duplex Target:** 5' AGAGAAGGGAGAAGAGAAAGAAATTTTTTTTTT 3' (SEQ ID NO:52)

3' TCTCTTTTTTCTTCTTTTCTTTAAAAAAAAA 5' (SEQ ID NO:53)